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Optical-Absorption Spectra of Ketyl Radicals and Radical Anions of Some Pyrimidines

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The pulse radiolysis of aqueous solutions of various pyrimidines has been studied under conditions where only solvated electrons are made to react with them. Transient optical-absorption spectra in the uv range have been obtained which provide information on the site of attack of e^-_{aq} on the pyrimidine molecule. These spectra have been identified as the ketyl radicals of the C_2 and C_4 carbonyls, based on examination of substituted pyrimidine derivatives and making use of the keto–enol tautomerism which these molecules undergo. At pH5.0 e^-_{aq} add at both the C_2 and C_4 positions, and the intermediates are rapidly protonated to give the corresponding ketyl radicals. The ketyl radicals dissociate to the corresponding ketyl radical anions with a $pK\sim 7.0$–7.5. In alkaline solutions, on ionization of one of the chromophores the ketyl radical anion of the other nondissociated carbonyl is formed. The ketyl radicals were found to have an extinction coefficient of $\sim 1000\ M^{-1}\cdot cm^{-1}$ and to decay by a second-order process with $2k\sim 10^9\ M^{-1}\cdot sec^{-1}$.

INTRODUCTION

Knowledge of the character of the electronic transitions responsible for all the absorption bands of pyrimidines and purines is only partially available.[1,2a] One of the primary features of these molecules is that most of them contain ionizable chromophores, so that variation and control of pH is of great importance in the spectroscopic elucidation of the molecular structures. However, in the study of the photochemistry[2] and radiation chemistry[3] of pyrimidines in aqueous solutions little attention seemed to have been placed on this particular feature to explain the effects observed. Tentative interpretations have been offered (for reviews, see Refs. 2 and 3) based almost exclusively on the reactivity of the 5,6 carbon–carbon double bond of the pyrimidine molecule, which quantum-chemical calculations have shown to be the most reactive site on the molecule.[1b] No serious consideration had been given to interpret results as possibly due, in part, to the ionizable carbonyls at positions C_2 and C_4 in the molecule.

In the course of studying the pulse radiolysis of pyrimidines in aqueous solutions,[4,5] some *relatively* weak transient absorptions were observed in the wavelength region 280–350 nm, which disappeared in the presence of an effective electron scavenger, such as nitrous oxide gas:

$$H_2O \rightsquigarrow e_{aq}^-,\ H,\ OH,\ H_2,\ H_2O_2,$$

$$e_{aq}^- + N_2O \rightarrow OH + N_2 + OH^-.$$

The observation of this transient species was found to be pH dependent, and it was provisionally suggested[4] that it may be "the mononegative uracil radical anion." The study of these species was, however, made difficult by the relatively stronger absorption of the OH radical adducts of the pyrimidines over the same wavelength region.

An OH radical scavenger was looked for which would not give rise to a transient absorption over the required wavelength range and which would be relatively unreactive towards the pyrimidines. The best choice was terbutanol which was found[6] to produce $\cdot CH_2(CH_3)_2$–COH radical with $\lambda_{max}=225$ nm, an $\epsilon_{225}=900 M\cdot cm^{-1}$ and $\epsilon_{280}=30 M\cdot cm^{-1}$, and a $k(OH+t\text{-BuOH})=2.5\times 10^8 M\cdot sec^{-1}$.[7]

The results presented below provide strong evidence for the ketonic properties of pyrimidines. These react with solvated electrons, mainly at the C_2- and C_4-carbonyl positions, to produce the corresponding ketyl radicals and radical anions, which have characteristic transient optical-absorption spectra. On ionization of one of the chromophores, only the absorption spectrum due to the other carbonyl–ketyl radical is observed.

EXPERIMENTAL

The pulse radiolysis of aqueous solutions of pyrimidines was carried out using a Febetron 705 system, which provides single pulses of electrons of 2.3 MeV energy and about 30-nsec duration. Rectangular through-flow Spectrosil quartz optical cells were used: 6 mm deep, 8 mm high, and 20 mm optical path. The thickness of the cell wall facing the electron beam was 0.5 mm.

The monitoring light source used was an Osram XBO 450W xenon lamp. The magnetic field from the Febetron interfered with the arc of the lamp. This was eliminated by shielding the lamp with consecutive

[1] (a) G. H. Beaven, E. R. Holiday, and E. A. Johnson, in *The Nucleic Acids*, E. Chargaff and J. N. Davidson, Eds. (Academic Press Inc., New York, 1955), Vol. 1. (b) B. Pullman and A. Pullman, *Quantum Biochemistry* (Interscience Publishers, Inc., New York, 1963).

[2] (a) A. D. McLaren and D. Shugar, *Photochemistry of Proteins and Nucleic Acids* (Pergamon Press Ltd., London, 1964). (b) J. G. Burr in *Advances in Photochemistry*, W. A. Noyes, Jr., G. S. Hammond, and J. N. Pitts, Jr., Eds. (Interscience Publishers, Inc., New York, 1968), Vol 6.

[3] G. Scholes, Progr. Biophys. Chem. **13**, 59 (1963). J. Weiss, Progr. Nucleic Acid Res. Mol. Biol. **3**, 103 (1964).

[4] R. M. Danziger, E. Hayon, and M. E. Langmuir, J. Phys. Chem. **72**, 3842 (1968).

[5] E. Hayon and R. M. Danziger (unpublished).

[6] M. Simic, P. Neta, and E. Hayon, J. Phys. Chem. (to be published).

[7] M. Anbar and P. Neta, Intern. J. Appl. Radiation Isotopes **16**, 227 (1965).

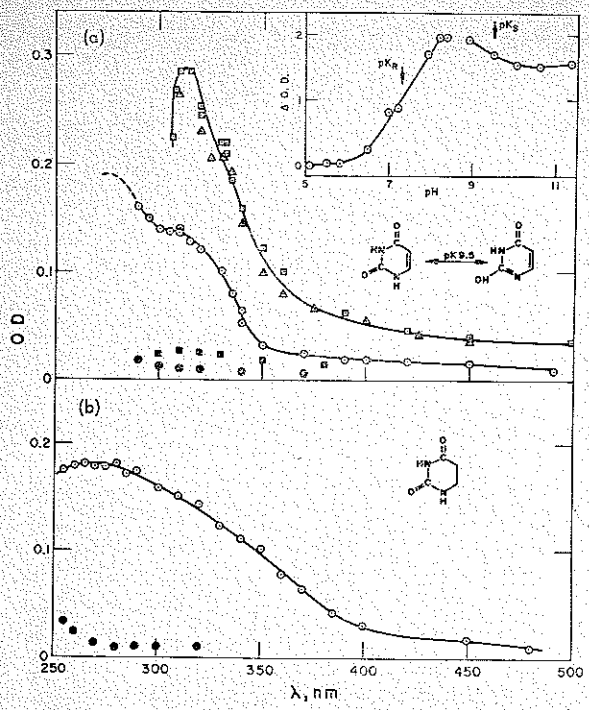


FIG. 1. Optical-absorption spectra obtained on pulse radiolysis of 0.5M t-BuOH containing (a) $2\times10^{-3}M$ uracil at pH 5.2 (⊙ Ar and ● N_2O), pH 11.7 (⊡ Ar and ■ N_2O) and in 0.2N NaOH (△). Insert: pK of radical as monitored at 330 nm (b) $2\times10^{-3}M$ dihydrouracil at pH 5.2 (⊙ Ar and ● N_2O) after correction for absorption below 280 nm of the t-BuOH radical.

layers of netic and conetic metal. Two high intensity Bausch & Lomb monochromators were used in series to reduce scattered light. EMI 9558 QB photomultipliers were used, and the photomultiplier and amplifier circuit has been described elsewhere.[8a]

Dosimetry was carried out[6] using N_2O-saturated 0.1M thiocyanate solutions at pH 5.5, by following the $(CNS)_2^-$ radical anion at 500 nm, taking[8b] $\epsilon_{500}=7600$ $M^{-1}\cdot cm^{-1}$. Total doses varying from ~8.0 to 36.5 krad/pulse were used in this work, based on a $g(e^-)+g(OH)=5.5$ The extinction coefficients of the various ketyl radicals observed were derived taking $g(e^-)=2.75$, assuming that all the e_{aq}^- were scavenged and react with the carbonyl chromophores In all cases where the ketyl radicals of the C_2- and C_4- carbonyls are produced simultaneously, i.e., at pH well below the first dissociation constant pK_1 of the pyrimidines, equal formation of each ketyl radical has been assumed. All the pK values used were obtained from Ref. 9.

Excess concentrations of pyrimidines were used to assure complete scavenging of e_{aq}^- and compete with the $e_{aq}^-+e_{aq}^-$ radical–radical combination reaction. For this purpose the rates of reactions of $k(e^-+$pyrimidine) were obtained from Refs. 7 and 10. Under the conditions used all the H atoms reacted with themselves and did not give rise to transient absorptions.

In all experiments carried out, 0.5M t-butanol was added to assure complete competition with the pyrimidines for the OH radicals produced in the radiolysis of water. To ascertain that the transient absorption spectrum observed is due to reaction of e_{aq}^- with pyrimidines, in every case the same solution was pulsed in presence of N_2O (1 atm). However, to simplify presentation of the results, the OD obtained in N_2O solutions is shown in only a few diagrams, but in all cases the transient absorption in N_2O was less than one-tenth of that in Ar-saturated solution. Due to the high extinction coefficients of some of the pyrimidines below 290 nm, it was not possible in some cases to obtain the complete absorption spectrum of the transient species in the far-uv range.

Reaction-rate constants were determined using a computer by least-squares approximation of first- and second-order reactions. The oscilloscope traces were read on a Gerber scanner (Model S-10-C), the data punched directly into cards, and rates calculated on a GE 225 computer.

The pH was varied using perchloric acid and sodium hydroxide. Near-neutral pH borates and phosphates were used as buffers. All reagents were best available from Cyclochemical, Calbiochem, and Mallinckrodt.

RESULTS AND DISCUSSION

The reaction of solvated electrons e_{aq}^-, produced from the radiolysis of water, with various substituted pyrimidine derivatives has been studied with the object of elucidating and determining the reactive site(s) of attack on the pyrimidine molecular structure. The approach was to use the technique of pulse radiolysis to observe the transient optical-absorption spectra of the "electron adducts" produced. The reaction-rate constants $k(e^-+$pyrimidine) had previously been measured[7,10] for most of the pyrimidines examined here and were found to be almost diffusion controlled (in the range about 2×10^9–$2\times10^{10}M^{-1}\cdot sec^{-1}$). The rate constants were also found to be lower in alkaline solutions, and Greenstock *et al.*[10] showed that this decrease followed the dissociation which these molecules undergo in the same pH range. Since all the pyrimidines studied exhibit ionizable chromophores, the effect of pH on the formation of the transient species was examined. To remove the strong interference caused in the same wavelength region by the transient absorption of the OH-radical adducts of these pyrimidines,[4,5,11] OH radicals were scavenged by adding 0.5M t-butanol.

[8] (a) J. P. Keene, E. D. Black, and E. Hayon, Rev. Sci. Instr. **40**, 1199 (1969). (b) J. H. Baxendale, P. L. T. Bevan, and D. A. Scott, Trans. Faraday Soc. **64**, 2389 (1969).

[9] (a) *The Pyrimidines*, D. J. Brown, Ed. (Interscience Publishers, Inc., 1962). (b) *Physical Methods in Heterocyclic Chemistry*, A. R. Katritzky, Ed. (Academic Press, Inc., New York, 1963), Vol. 1.

[10] C. L. Greenstock, M. Ng. Hunt, and J. W. Hunt, Advan. Chem. Ser. **81**, 397 (1968).

[11] L. S. Myers, Jr., M. L. Hollis, and L. M. Theard, Advan. Chem. Ser. **81**, 345 (1968).

The physical and chemical properties[6] of the $\cdot CH_2(CH_3)_2$ COH radical produced makes this alcohol a desirable additive in pulse radiolysis studies of e_{aq}^- with solutes and has been considerably used for this purpose in this laboratory.

URACIL, THYMINE, AND N-METHYL DERIVATIVES

The transient optical-absorption spectra obtained on pulse radiolysis of O_2-free $2\times10^{-3}M$ uracil in presence of $0.5M$ *t*-BuOH at *p*H 5.2 and *p*H 11.7 are shown in Fig. 1(a). In neutral solution two transient species are formed with $\lambda_{max}\sim305\pm5$ nm and <280 nm. On saturating the solution with N_2O, this transient spectrum disappears completely, and no other absorption can be seen in the wavelength region 280–800 nm [Fig. 1(a)]. At *p*H greater than the dissociation constant $pK_1=9.5$ of uracil, only one transient species is observed on pulse radiolysis at *p*H 11.7, Fig. 1(a), with $\lambda_{max}\sim310\pm5$ nm. It is worth noting the OD of the transient in alkaline solutions, when the C_2-carbonyl is present in the enolic form, is about double that obtained at *p*H 5.5:

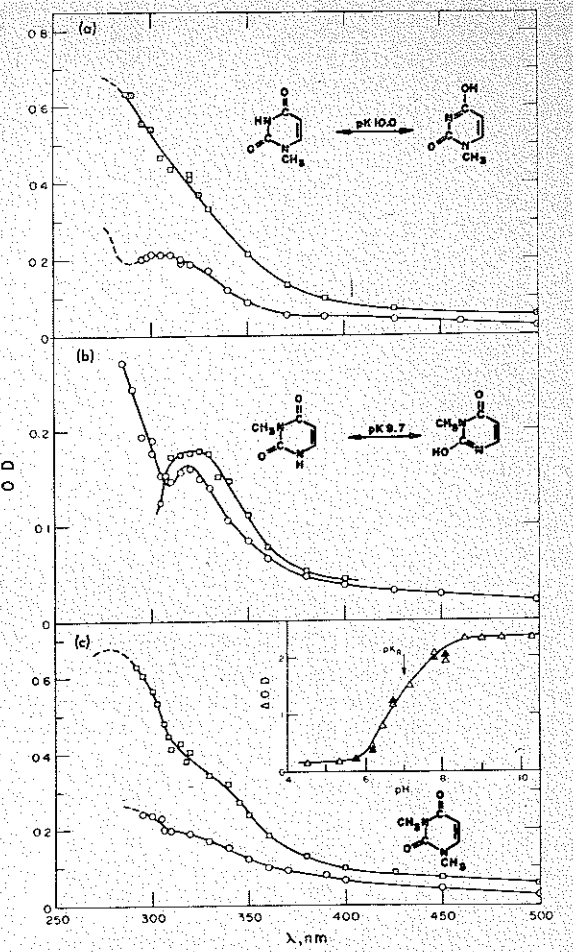
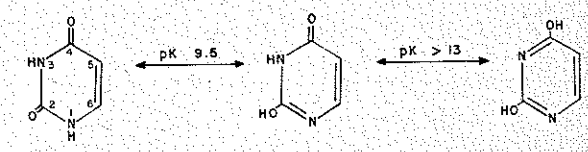


FIG. 3. Optical-absorption spectra obtained on pulse radiolysis of Ar-saturated $0.5M$ *t*-BuOH containing (a) 1-methyluracil at *p*H 5.3 (⊙) and *p*H 11.8 (⊡); (b) $2\times10^{-3}M$ 3-methyl uracil at *p*H 6.0 (⊙) and *p*H 11.8 (⊡); (c) $2\times10^{-3}M$ 1,3-dimethyluracil at *p*H 5.1 (⊙) and *p*H 12.4 (⊡). Insert is *pK* of radical as monitored at 300 nm (△) and 340 nm (▲).



and the transient absorption maximum at <280 nm is not present. For simplification, the hydroxyl groups in this paper are not presented in their dissociated $-O^-$ forms. In $0.2N$ NaOH, no apparent change is observed [Fig. 1(a)] indicating that the C_4 carbonyl is not yet dissociated at this OH^--ion concentration. On pulse radiolysis of dihydrouracil a maximum at 275 ± 5 nm is obtained [Fig. 1(b)], with indications (not shown) of another transient absorption band below 250 nm. Due to the instability of dihydrouracil in alkaline solutions it was not possible to study it at high *p*H. The presence of a transient species produced from the reaction of e_{aq}^- with dihydrouracil provides strong

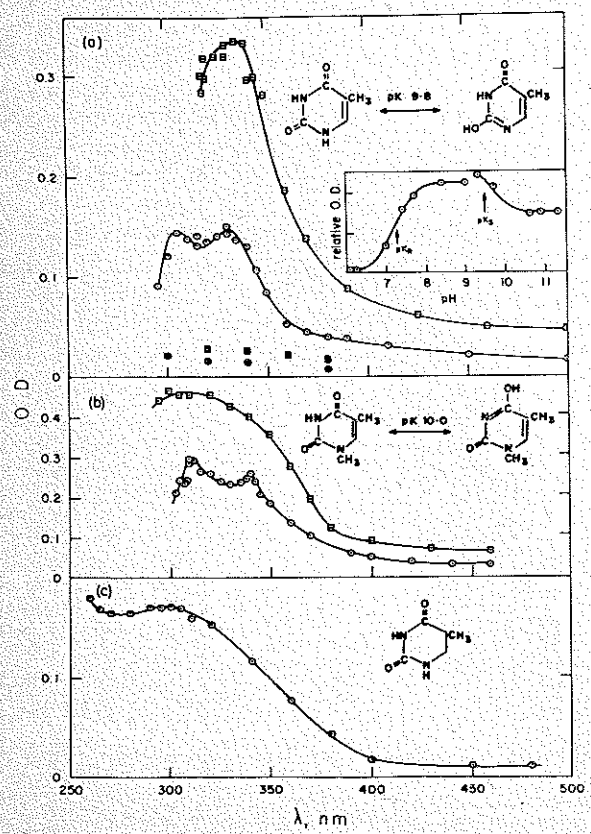


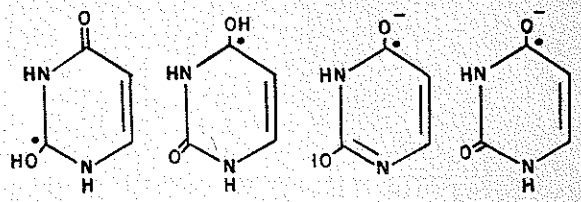
FIG. 2. Optical-absorption spectra obtained on pulse radiolysis of $0.5M$ *t*-BuOH solutions containing (a) $2\times10^{-3}M$ thymine at *p*H 5.1 (⊙ Ar and ● N_2O), and *p*H 11.8 (⊡ Ar and ■ N_2O). Insert: *pK* of radical monitored at 340 nm; (b) $2\times10^{-3}M$ 1-methylthymine at *p*H 5.5 (⊙ Ar) and *p*H 12.1 (⊡ Ar); (c) $2\times10^{-3}M$ dihydrothymine at *p*H 5.0 (⊙ Ar).

support (see also below) to the proposal made in this paper that e_{aq}^- react mainly with the C_2 and C_4 carbonyls and not with the 5,6 carbon–carbon double bond, as had been postulated previously.[3,12,13]

The transient absorption produced from the reaction of e_{aq}^- with thymine at *p*H 5.1 is shown in Fig. 2(a), with maxima at ~330 and ~305 nm. On pulse radiolysis at *p*H 11.8, only one transient is formed at λ_{max} ~330 nm. On irradiation of 1-methylthymine at *p*H 5.5, two maxima are observed at ~340 and ~310 nm; at *p*H 12.1, only one maximum at 310 nm is found [Fig. 2(b)]. On methylation of the N_1 position, the dissociation takes place at the C_4-carbonyl position, and one must note that in this case the far-uv maximum at the C_2 carbonyl position only is observed, contrary to what was found for uracil and thymine. Figure 2(c) shows the transient absorption found on pulsing dihydrothymine at *p*H 5.0; in alkaline solutions it is unstable and decomposes.

The absorption spectra obtained on pulse radiolysis of 1-Me-, 3-Me-, and 1,3-dimethyluracils at neutral and alkaline *p*H are shown in Fig. 3. In these three N–methyluracils two peaks are produced in neutral solution (the maxima of the far-uv peaks could not be established accurately due to the strong absorption by the parent compounds); in alkaline solution only one peak is obtained for N_1–methyl and N_3–methyluracils. No change in the absorption spectrum is found in alkaline solutions of 1,3-dimethyluracil (see also below), in agreement with the fact that this molecule has no ionizable chromophore.

Before proceeding further and presenting the results obtained with other pyrimidines, the results given above will be discussed. The following points can be made: (a) all the transient optical absorption spectra obtained result from the reaction of e_{aq}^- with the various pyrimidines, not from reaction of OH radicals or *t*-butanol radicals, as evidenced by the absence of the spectra in N_2O (1 atm) solutions; (b) the transient species are not produced by reaction of e_{aq}^- at the 5,6 carbon–carbon double bond since similar spectra are obtained from dihydrouracil and dihydrothymine (one cannot exclude entirely a *small* percentage of the electrons reacting by adding to the double bond). The shift in the position of the transient absorption maxima follows the shift in the absorption spectra of the dihydro derivatives to lower wavelengths; (c) the first *pK* of these pyrimidines has been shown[2a,9] to be due to the dissociation at the C_2 position, except when the N_1 position is methylated in which case dissociation at the C_4 position takes place. The change in the transient absorption spectra on pulse radiolysis of these pyrimidines in alkaline solutions is consistent with the ionization of the carbonyl chromophore at the C_2 position (or at C_4 position in the case of 1-methylthymine and 1-methyluracil); (d) in all cases (see also below) the far-uv absorption maxima is consistent with the addition of the electron at the C_2-carbonyl position, and the near-uv absorption maxima to the addition at the C_4-carbonyl position. For example in neutral solution of uracil two ketyl radicals are formed (I), (II), but in alkaline solutions (up to 0.3*N* NaOH) only one ketyl radical (III) is formed:



λ_{max} <280nm λ_{max} ~305nm λ_{max} ~310nm

(I) (II) (III) (IV)

On methylation of the N_1, N_3, or C_5 positions, the maxima of the transient species observed in neutral and alkaline solutions are consistent with the absorption peak at longer wavelength always due to the ketyl radical at the C_4 position. In the case of 1,3-dimethyluracil, ketyls at the C_2 and C_4 positions are formed in both neutral and alkaline solutions; (e) from the change in OD of the maxima in alkaline compared to neutral solutions, it would appear that in most cases there is an equal reactivity for the e_{aq}^- at the C_2 and C_4 positions, but the extinction coefficient of the C_2–ketyl radical is, in some cases, somewhat higher than that of the C_4–ketyl radical.

To determine whether the transient spectra obtained at *p*H 5.0–5.5 are due to the ketyl radicals or to the ketyl radical anions, the change in absorption was monitored at a fixed wavelength, as a function of *p*H, to obtain the dissociation constant of these species. The

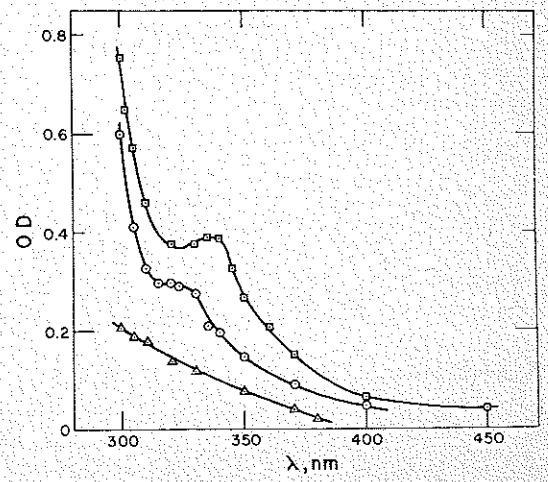


FIG. 4. Optical-absorption spectra obtained on pulse radiolysis of 0.5*M* *t*-BuOH, borate buffer, containing $2\times10^{-3}M$ uracil (⊙, at *p*H 8.2), thymine (⊡, at *p*H 8.2), and cytosine (△, at *p*H 9.2).

[12] C. Nofra and A. Cier, in *Electronic Aspects of Biochemistry*, B. Pullman, Ed. (Academic Press Inc., New York, 1964).
[13] A. Kamal and W. M. Garrison, Nature **206**, 1315 (1965).

inserts in Figs. 1(a), 2(a), and 3(c) show the change observed in uracil, thymine, and 1,3-dimethyluracil. In the case of uracil and thymine, two dissociations are found, the first one due to the dissociation of the ketyl radical to the radical anion at a pK 7.3±0.2 and 7.2±0.3, respectively, and the second dissociation due to the keto–enol tautomerism of the C_2-carbonyl chromophore at pK 9.5 and 9.8, respectively. It can be seen that the extinction coefficient of the radical anions (IV) are greater by a factor of 2–4 as compared to (II), while the extinction coefficient at a $p\mathrm{H} > pK_1$ of the C_4-ketyl radical anion (III) is very close to (II). This increase in the extinction coefficient of the ketyl radical anion, compared to the undissociated ketyl radical, is similar to that found for aromatic and aliphatic[6] ketyl radicals. Dimethyluracil undergoes no dissociation, and the change observed [Fig. 3(c)] is due to the increase in extinction coefficient of the ketyl radical anion, with $pK_R = 7.0 \pm 0.2$.

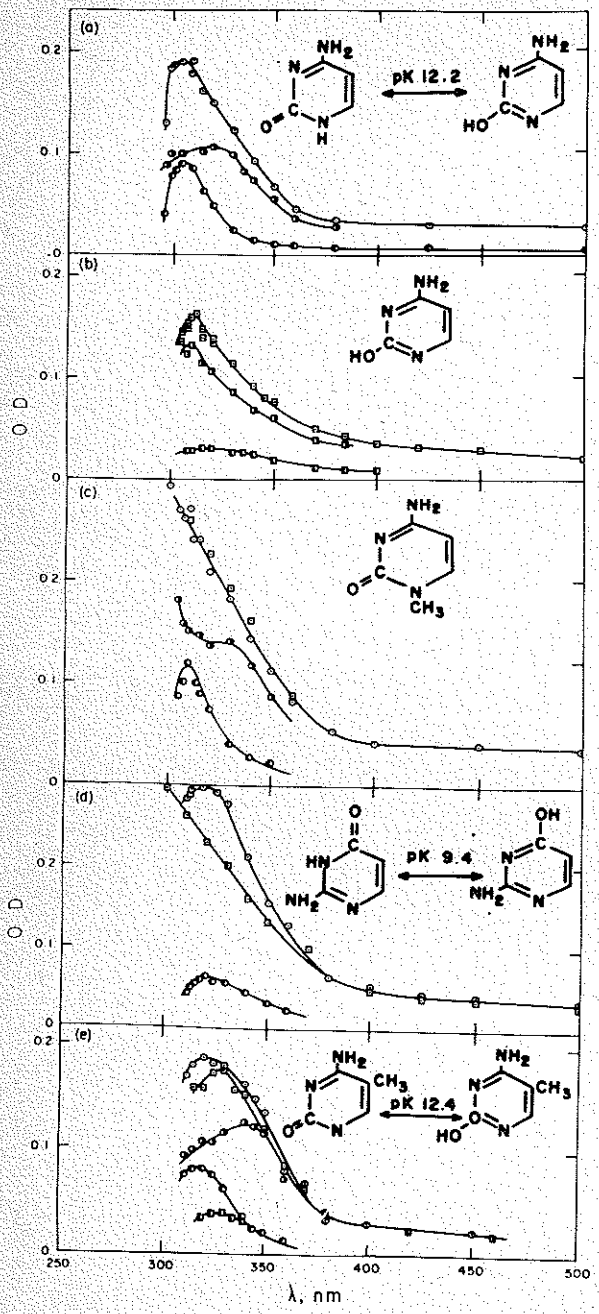


FIG. 5. Optical-absorption spectra obtained on pulse radiolysis of Ar-saturated 0.5M t-BuOH containing (a) $2\times10^{-3}M$ cytosine at pH 5.5 read at ≤1 μsec (⊙), at 30 μsec (◐), and difference (◑); (b) $2\times10^{-3}M$ cytosine in 0.2N NaOH read at ≤1 μsec (⊡), at 30 μsec (◧), and difference (◨); (c) $2\times10^{-3}M$ 1-methylcytosine at pH 5.7 read at ≤1 μsec (⊙), at 30 μsec (◐), and difference (◑), and at pH 11.8 (⊡); (d) $2\times10^{-3}M$ isocytosine at pH 7.5 read at ≤1 μsec (⊙) and 30 μsec (◐) and pH 11.5 (⊡); (e) $2\times10^{-3}M$ 5-methylcytosine at pH 5.2 read at ≤1 μsec (⊙), at 30 μsec (◐), and difference (◑), and at pH 13.4 read at ≤1 μsec (⊡) and 30 μsec (◧).

The transient absorption spectra of the C_2– and C_4–ketyl radical anions of uracil (at pH 8.20) and thymine (at pH 8.20) are shown in Fig. 4. One can note in Fig. 4 that the maxima of the C_2– and C_4–ketyl radical anions are shifted away from each other, and that the extinction coefficient of the C_2–ketyl radical anions is considerably increased. Values for the extinction coefficients, decay constants, and suggested structure of the transient species observed are given in Table I.

In all cases, the decay rate constants are lower in alkaline solutions, compared to the decay of the radicals at pH 5.0–5.5. This is as expected since in alkaline solution the combination reaction is between negatively charged species, while at pH 5.0 the radicals and the pyrimidines are undissociated. All the decay constants given in Table I are subject to a possible error of 20%–30%, due to the probable partial interaction between the ketyl radical anions and the t-butanol radical. The decay constant of the t-butanol radical was found[6] to be $2k = 1.3\times10^{9}M^{-1}\cdot\mathrm{sec}^{-1}$. One must also consider the possibility that the t-butanol radical may add to the 5,6 carbon–carbon double bond of the pyrimidine molecule. Such an addition was found[14] for the CH_3CHOH radical to thymine.

CYTOSINE AND ISOCYTOSINE

Figures 5(a) and 5(b) show the transient spectra obtained on pulse radiolysis of O_2-free solutions of cytosine at pH 5.5 and in 0.2N NaOH. Two transients are produced in neutral solution, one with a lifetime of ~30 μsec and a maximum at ~320±10 nm and the other with a lifetime ≤100 μsec peaking at ~305±5 nm. In alkaline solution, one main transient is apparently formed, λ_{max} ~310 nm. Somewhat similar results are obtained with 1-Me-cytosine [Fig. 5(c)] in neutral solution. In alkaline solutions, no discernable difference was found. On pulse radiolysis of isocytosine in neutral solution, Fig. 5(d), a transient with λ_{max} ~320 nm was

[14] P. E. Brown, M. Calvin, and J. F. Newmark, Science 151, 68 (1966).

TABLE I. Absorption maxima, extinction coefficients, and decay rate constants of ketyl radicals and radical anions of some pyrimidines in aqueous solution.

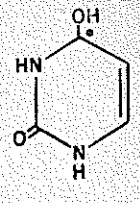
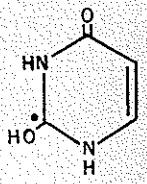
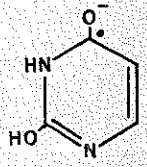
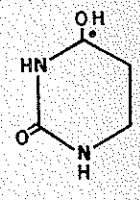
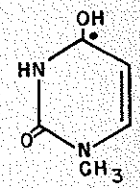
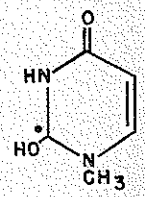
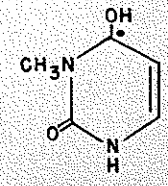
System	*p*H	λ_{max}(nm)	$\epsilon(M^{-1}\cdot cm^{-1})$ [a]	Decay constant $\times 10^8\ M^{-1}\cdot sec^{-1}$ [b]	Suggested species
Uracil	5.1	305±5	1.4×10^3	35	
	5.1	<280	...	...	
	11.7; 0.3*N* NaOH	310±5	1.5×10^3	9	
Dihydrouracil	5.2	275±5	1.8×10^3	29	
1-Me-uracil	5.3	305±5	2.2×10^3	42	
	5.3	<280	...	...	
3-Me-uracil	6.0	320±5	1.4×10^3	34	

TABLE I (*Continued*)

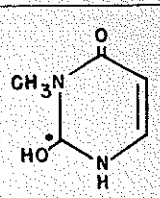
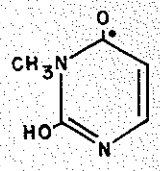
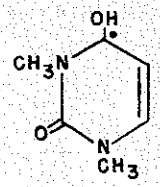
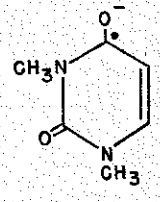
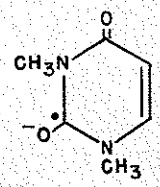
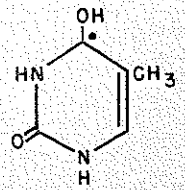
System	pH	λ_{max}(nm)	$\epsilon(M^{-1}\cdot cm^{-1})$[a]	Decay constant $\times 10^5\ M^{-1}\cdot sec^{-1}$[b]	Suggested species
	6.0	<285	...	...	
	11.8	320±5	1.0×10^3	10	
1:3 Dimethyl-uracil	5.1	~320	1.9×10^3	75	
	12.4	~325	3.6×10^3	20	
	12.4	<290	6.0×10^3 at 295 nm	15	
Thymine	5.1	330±3	1.5×10^3	65	

TABLE I (*Continued*)

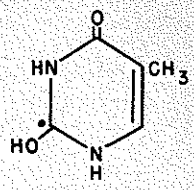
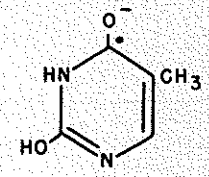
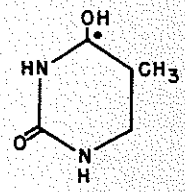
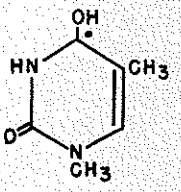
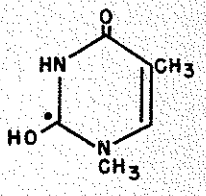
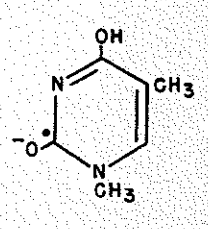
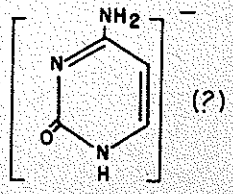
System	*p*H	λ_{max}(nm)	$\epsilon(M^{-1}\cdot cm^{-1})$ [a]	Decay constant $\times 10^8\ M^{-1}\cdot sec^{-1}$ [b]	Suggested species
	5.1	305±3	1.5×10^3	50	
	11.8	330±5	1.7×10^3	12	
Dihydrothymine	5.0	290±5	1.6×10^3	...	
1-Me-thymine	5.5	340±3	2.3×10^3	35	
	5.5	310±3	2.5×10^3	42	
	12.1	310±5	1.3×10^3	7	
Cytosine	5.5	320±10	...	2×10^6 [c]	

TABLE I (*Continued*)

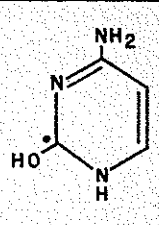
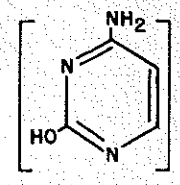
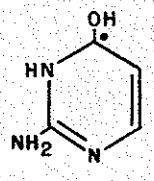
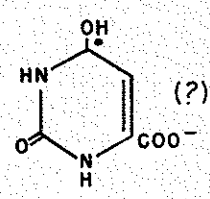
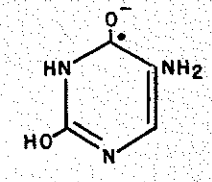
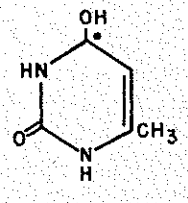
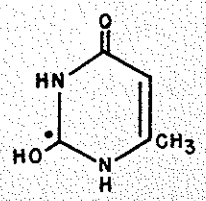
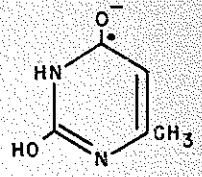
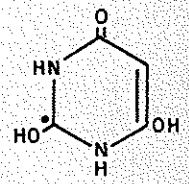
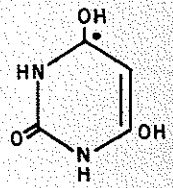
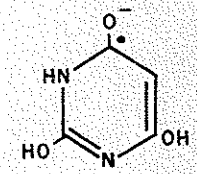
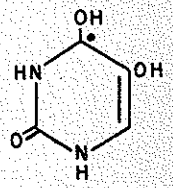
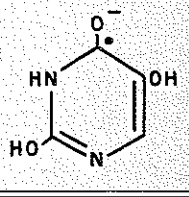
System	pH	λ_{max}(nm)	$\epsilon(M^{-1}\cdot cm^{-1})$ [a]	Decay constant $\times 10^8\ M^{-1}\cdot sec^{-1}$ [b]	Suggested species
	5.5	305±5	...	8×10^5 [c]	 (?)
	13.3	310±5	8×10^2	14	 (?)
Isocytosine	7.5	317±5	1.5×10^3	15	 (?)
Orotic acid	5.8	328±5	1.3×10^4	7	 (?)
5-Aminouracil	11.7	355±5	1.6×10^3	16	
6-Me-uracil	5.3	325±5	1.2×10^3	45	
	5.3	295±3	1.3×10^3	50	

TABLE I (*Continued*)

System	*p*H	λ_{max} (nm)	$\epsilon(M^{-1}\cdot cm^{-1})$ [a]	Decay constant $\times 10^8\ M^{-1}\cdot sec^{-1}$ [b]	Suggested species
	11.8	310±5	0.9×10^3	9	
Barbituric acid	5.0	310±10	1.4×10^3	14	
	5.0	420±10	9.0×10^2	8	
	12.9	420±10	7×10^2	7	
Isobarbituric acid	5.0	340±5	2×10^3	20	
	12.2	340±5	1.8×10^3	6	

[a] Deviation ±20%; in neutral solution when both C_2– and C_4–ketyl radicals are formed and ϵ is estimated, deviation is ±50%.

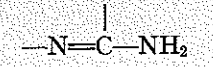
[b] Deviation less than ±20%.

[c] $2k/\epsilon$ decay rate values.

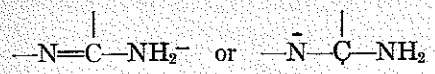
obtained. This is suggested to be the C_4–ketyl radical of isocytosine. In alkaline solutions, a different transient is produced having a maximum below 290 nm and is considered to be formed by the addition of e_{aq}^- at either the N_1 or C_2 positions—possibly on the latter group. The radiolysis of 5-methylcytosine gives two transient species, Fig. 5(e), as obtained for cytosine and 1-Me-cytosine. However, as seen above, a methyl group at the C_5 position of uracil shifts the position of the maxima to higher wavelengths.

The results obtained in Fig. 5 are more difficult to interpret, and more work is under way. It is clear, how-

ever, that one of the transients is due to the ketyl radical and the other is connected with the



group and could be either the



mononegative radical anion. Support for the latter radical is derived from the transient produced from the reaction of e_{aq}^- with 4-hydroxypyrimidine in alkaline solution (see below). In alkaline solution, only the mononegative radical anion is formed. If the cytosines behave similarly to the other pyrimidines, the maximum at higher wavelength could be the radical produced at the C_4 (or N_3) position, and that at lower wavelength to the radical at the C_2 (or N_1) position.

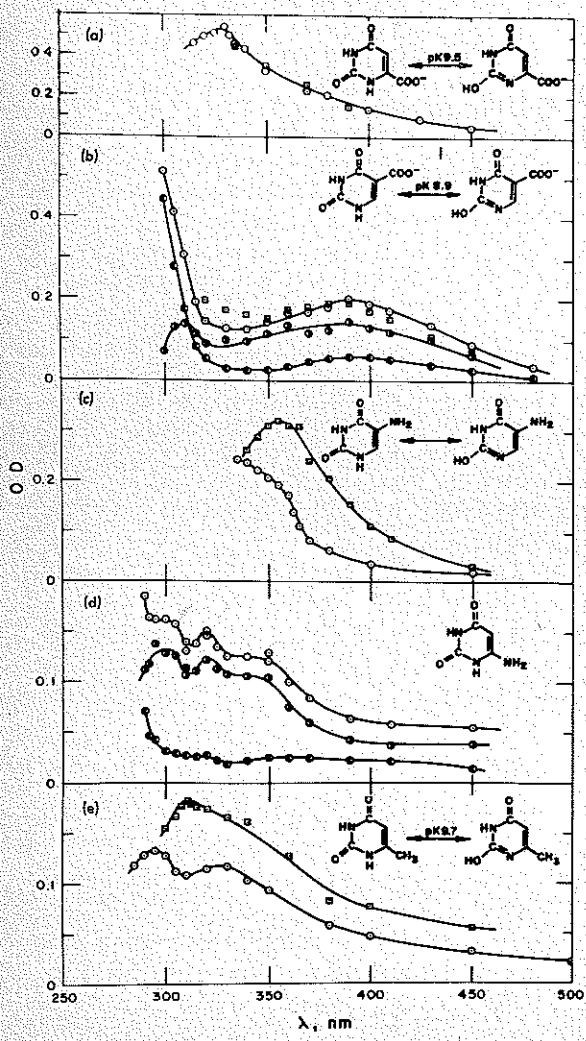


FIG. 6. Optical-absorption spectra obtained on pulse radiolysis of Ar-saturated 0.5M t-BuOH containing (a) $2\times10^{-3}M$ orotic acid at pH 5.8 (⊙) and pH 11.6 (⊡); (b) $2\times10^{-3}M$ iso-orotic acid at pH 5.8 read at ≤1 μsec (⊙), at 50 μsec (◐), and difference (◑), and at pH 11.6 (⊡); (c) $2\times10^{-3}M$ 5-aminouracil at pH 5.6 (⊙) and pH 11.7 (⊡); (d) 6-aminouracil at pH 6.8 read at ≤1 μsec (⊙), at 60 μsec (◐), and difference (◑); (e) $2\times10^{-3}M$ 6-methyluracil at pH 5.3 (⊙) and pH 11.8 (⊡).

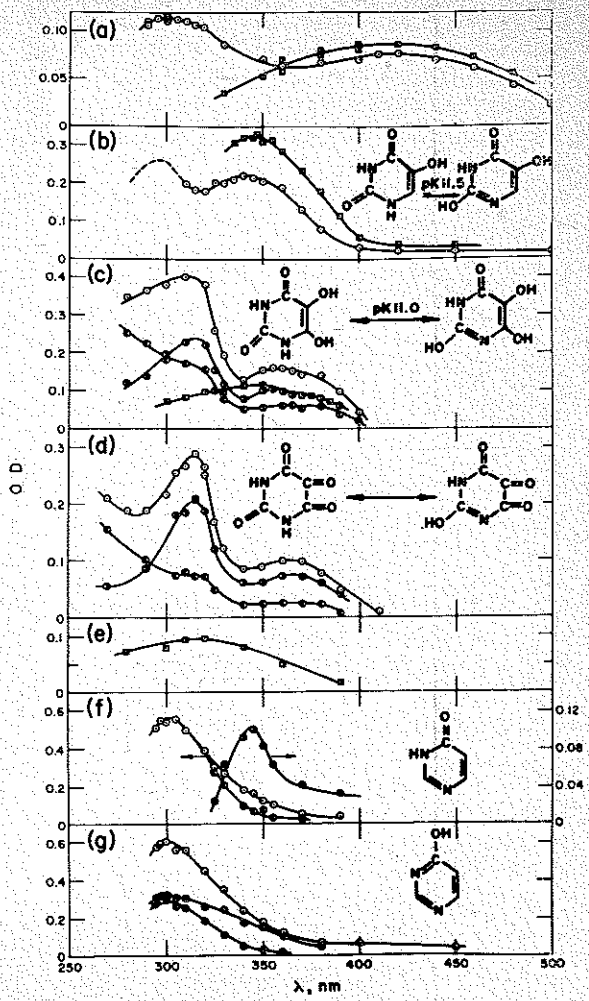


FIG. 7. Optical-absorption spectra obtained on pulse radiolysis of Ar-saturated 0.5M t-BuOH solutions containing (a) $2\times10^{-3}M$ barbituric acid at pH 5.0 (⊙) and pH 12.9 (⊡); (b) $2\times10^{-3}M$ isobarbituric acid at pH 5.0 (⊙) and pH 12.2 (⊡), dotted lines are corrected data; (c) $2\times10^{-3}M$ dialuric acid at pH 5.5 read at ≤1 μsec (⊙), at 40 μsec (◐), and difference (◑), also $2\times10^{-3}M$ dialuric acid at pH 12.1 (⊡); (d) $2\times10^{-3}M$ alloxan at pH 5.1 read at ≤1 μsec (⊙), at 300 μsec (◐), and difference (◑); (e) $2\times10^{-3}M$ alloxan at pH 12.1 (⊡); (f) $2\times10^{-3}M$ 4-hydroxyuracil at pH 5.2 read at ≤1 μsec (⊙) at 100 μsec (◐), and difference (◑); (g) $2\times10^{-3}M$ 4-hydroxyuracil at pH 12.1 read at ≤1 μsec (⊙), at 100 μsec (◐), and difference (◑).

C_5–C_6– SUBSTITUTED DERIVATIVES

The optical-absorption spectra of the ketyl radical and radical anions formed on pulse radiolysis of orotic acid, iso-orotic acid, 5-aminouracil, 6-aminouracil, and 6-methyluracil are shown in Fig. 6. The results obtained in some of these systems appear somewhat complicated, and further work will be undertaken to assign radicals to these spectra. The following points can, however, be made. It would seem, from the similarity of the spectrum at pH 5.8 and 11.6, that the main

ketyl radical of orotic acid is at the C_4 carbonyl position. In the case of iso-orotic acid, two maxima are observed (at $\sim$310±5 nm and $\sim$375±10 nm) and could be due to the C_2– and C_4–ketyl radicals, respectively. On pulse radiolysis of 5-aminouracil, maxima at 360±5 nm and <330 nm are produced, and these are considered to be the C_4– and C_2–ketyl radicals, respectively. The transient spectrum produced in 5-aminouracil at *p*H 11.7, Fig. 6(c), is in agreement with this postulate.

The spectra produced on radiolysis of 6-aminouracil are more complex and could be due to the proximity of the maxima of the two ketyl radicals. The irradiation of 6-methyluracil gives a spectrum, Fig. 6(e), somewhat similar to that obtained from thymine [see Fig. 2(a)]. The apparent maxima at 325±5 nm and 295±5 nm at *p*H 5.3 are due to the C_4– and C_2–ketyl radicals, respectively, but due to a probable overlap of the spectra the position of these maxima (this is true for all the other spectra obtained in this work) may not be correct. Indeed, at *p*H 11.8 when only the C_4–ketyl radical anion of 6-methyluracil is produced, the absorption maxima appears at 310±5 nm.

In Fig. 7, the transient absorption spectra obtained on radiolysis of barbituric acid, isobarbituric acid, dialuric acid, alloxan, and 4-hydroxypyrimidine are shown. Again, in some cases, the spectra are complex and further work is needed to clarify the situation.

The results in barbituric and isobarbituric acids appear fairly clear. In the former case, maxima at 310±10 and 420±10 nm are interpreted as the ketyl radicals of barbituric acid at the C_2 and C_4 positions, respectively. In alkaline solutions, when the C_2 carbonyl is dissociated, only the C_4 ketyl is formed [Fig. 7(a)] with a broad maximum at 420±20 nm. With isobarbituric acid, the same is observed, Fig. 7(b), except that the position of the two maxima is shifted to lower wavelengths. These results have been of considerable help in identifying the intermediates produced in the flash photolysis of 5-bromouracil.[15] The results obtained in the pulse radiolysis of dialuric acid, Fig. 7(c), could be interpreted as due to the presence of two maxima, the C_2– and C_4–ketyl radicals—but the presence of a strongly absorbing slow-decaying transient makes the identification of these maxima less certain. The same is true in the case of alloxan, Fig. 7(d).

The pulse radiolysis of 4-hydroxypyrimidine at *p*H 5.2 gives rise to two transient species; one with a maximum at 340±5 nm and provisionally assigned to the C_4–ketyl radical of pyrimidine; the other with λ_{max} $\sim$300 nm to an electron adduct. A similar species appears on radiolysis at *p*H 12.1 (the *pK* of 4-hydroxypyrimidine is 8.60) but no species with λ_{max} at 340 nm is observed. Since the 300-nm transient is not present in N_2O (1 atm), it is suggested to be produced as the result of reaction of e_{aq}^- with the N_1 or N_3 double bond. Current work[16] on the reactions of solvated electrons with *N*-heterocyclic compounds will, it is hoped, provide further information on the position of attack of e_{aq}^-.

CONCLUSIONS

These results provide for the first time information on the ketonic properties of uracil and its derivatives. The high reactivity of these molecules with solvated electrons can be seen to be due to the attack of e_{aq}^- at the C_2– and C_4–carbonyl positions, whenever possible. The ketyl radicals and ketyl-radical anions produced have characteristic optical absorption maxima and decay by second-order kinetics. Similar results[16] have been obtained with purines and other compounds of biological interest. The nature of the product(s) produced from the reaction of two ketyl radicals remains to be identified, and could be of considerable importance in the understanding of the effects of ionizing radiations on the pyrimidine and purine bases of DNA and RNA.

ACKNOWLEDGMENT

I would like to thank Mrs. L. M. Dogliotti for reading and programing the oscilloscope decay traces obtained in this paper.

[15] M. E. Langmuir and E. Hayon, J. Chem. Phys. 51, 4893 (1969), following paper.

[16] E. Hayon (unpublished).